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Abstract: Experiments in animal models have identified specific brain regions such as the deep anterior piriform cortex as important for controlling the initiation or propagation of both generalized and focal seizure activity. However, there is little experimental evidence to translate these observations to the control of focal seizures in humans. Here, we summarize findings from different hemodynamic and neurotransmitter functional imaging studies in groups of patients with focal epilepsies arising from different cortical locations in support of a common area of brain dysfunction in focal epilepsies.

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Functional Imaging of the Piriform Cortex in Focal Epilepsy

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Summary:

Experiments in animal models have identified specific brain regions such as the deep anterior piriform cortex as important for controlling the initiation or propagation of both generalized and focal seizure activity. However, there is little experimental evidence to translate these observations to the control of focal seizures in humans. We summarise findings from different hemodynamic and neurotransmitter functional imaging studies in groups of patients with focal epilepsies arising from different cortical locations in support of a common area of brain dysfunction in focal epilepsies.

Keywords: focal epilepsy, Flumazenil-PET, BOLD, EEG/fMRI, subcortical

Abbreviations: EEG, electroencephalography; MRI, magnetic resonance imaging; fMRI, functional MRI; EEG/fMRI, EEG combined with simultaneous fMRI; PET, positron emission tomography; FMZ, flumazenil; FMZVD, FMZ volume of distribution; IED, interictal epileptic discharge; GABA, gamma aminobutyric acid;

Introduction

During generalized epileptic seizures in animal models and human patients, electrical discharges oscillate between cerebral cortex, thalamus and basal ganglia (Depaulis et al., 1994; Deransart et al., 1998; Piredda and Gale, 1985; Steriade, 2005). During focal cortical seizure activity, specific cortical-subcortical circuits contribute to sustaining and propagating the seizure discharge. Experiments in animal models have identified specific brain regions such as the substantia nigra and the deep anterior piriform cortex as important for controlling the initiation or propagation of both generalized and focal seizure activity (Biraben et al., 2004; Bouilleret et al., 2005; Depaulis et al., 1994; Deransart et al., 1998; Piredda and Gale, 1985). In rat and monkey, a discrete site within the deep piriform (olfactory) cortex, termed *area tempestas* or "ventrostriatal anterior piriform cortex" is critical for modulating focal seizures (Ekstrand et al., 2001; Piredda and Gale, 1985). However, there is little experimental evidence to translate these observations to the human situation (Blumenfeld et al., 2004). Observations with deep brain stimulation in a variety of subcortical structures in patients with epilepsy (Morrell, 2006) suggest that the circuitry has the potential to be harnessed for therapeutic benefit.

Positron emission tomography

Akin to Karen Gale's experiments in non-human primates, we sought to characterize GABA-mediated mechanisms in humans *in vivo*, by performing a group analysis of carbon-11 labeled flumazenil (FMZ) positron emission tomography (PET) studies in 18 patients with different extra-temporal epilepsy syndromes. (Laufs et al, 2011) Compared to 24 controls, we found significant increases in FMZ volume of distribution (V_T) in the putamen bilaterally. Restricting our analysis only to those regions identified in the first analysis, we then asked whether this difference in FMZ-V_T can be explained by seizure-frequency as a measure of epilepsy severity. FMZ-V_T correlated negatively with seizure frequency over the preceding month in an area in the vicinity of the deep anterior piriform cortex, adjacent to putamen and claustrum, i.e. the higher the FMZ-V_T, the lower the seizure frequency.

Our results are concordant with chemical stimulation of this region in the deep piriform cortex through unilateral microinjection of a GABA receptor antagonist or glutamate receptor agonists, which triggered limbic motor seizures in the rat (Piredda and Gale, 1985; Doherty et al., 2000) and limbic seizures and status epilepticus in non-human primates (Gale, 1995; Gunderson et al., 1999). Local application of the GABA agonist muscimol prevented seizures, which would have occurred following microinjection of all convulsant agents examined

(Piredda and Gale, 1985). Immunostaining revealed particular features of this site that could alter excitability, including a near-absence of GABAergic "cartridge" endings on axon initial segments, and very low gamma-aminobutyric acid transporter-1 (GAT1)-like immunoreactivity. Normally, the function of this area may be to shape neuronal activity through inhibitory processes so that this region is no longer susceptible to pathological behaviour (Ekstrand et al., 2001). Our data of an increase in FMZ-V_T in this area and the observed association of increased binding with a reduced seizure frequency might reflect compensatory mechanisms of seizure suppression: postsynaptic increases in the number of $GABA_A$ receptors have been described in the kindling model underlying inhibitory potentiation (Nusser et al., 1998). Such an increase in post-synaptic binding sites will lead to a measurable increase in FMZ-V_T. Similarly, pre- and postsynaptic changes of GABA transmission involving changes of $GABA_A$ ß receptor subunit composition have been found using the pilocarpine model (Brooks-Kayal et al., 1998).

Of note, in an excellent FMZ PET study in patients who were monitored with video-EEG telemetry in the week before their PET scan as part of presurgical monitoring, the reduction of FMZ-V_T in the mesial temporal structures was greatest the closer the PET scan was performed following a seizure. (Bouvard et al., 2005) The authors did not specifically report on sub-cortical changes, but abnormalities can be seen on their figures not only in area of the piriform cortex, but also in the brainstem, possibly substantia nigra. Thus, we can speculate that the greater the increase in FMZ binding in the piriform cortex, the fewer the seizures, as observed in our study.

Functional Magnetic Resonance Imaging

Following the same principle as for the above described PET studies, we were the first to perform group analysis functional MRI (fMRI) studies with simultaneous electroencephalography (EEG) recording in patients with clear-cut focal epilepsies from a wide variety of cortical locations. (Laufs et al, 2011) EEG-fMRI measures haemodynamic changes correlated with a measure of epilepsy activity, i.e. the interictal epileptic discharge (IED). Following the assumption that increased spike frequency reflects more severe epilepsy, similar to the use of seizure-frequency as a covariate of interest for the FMZ PET analysis, our aim was to detect hemodynamic changes common to all patients, but independent of the site of focal IEDs.

We re-analysed IED correlated fMRI data in an event-related fashion from 19 patients with focal epilepsy, selected from a larger pool of 63 patients on the basis of a spiking rate in the

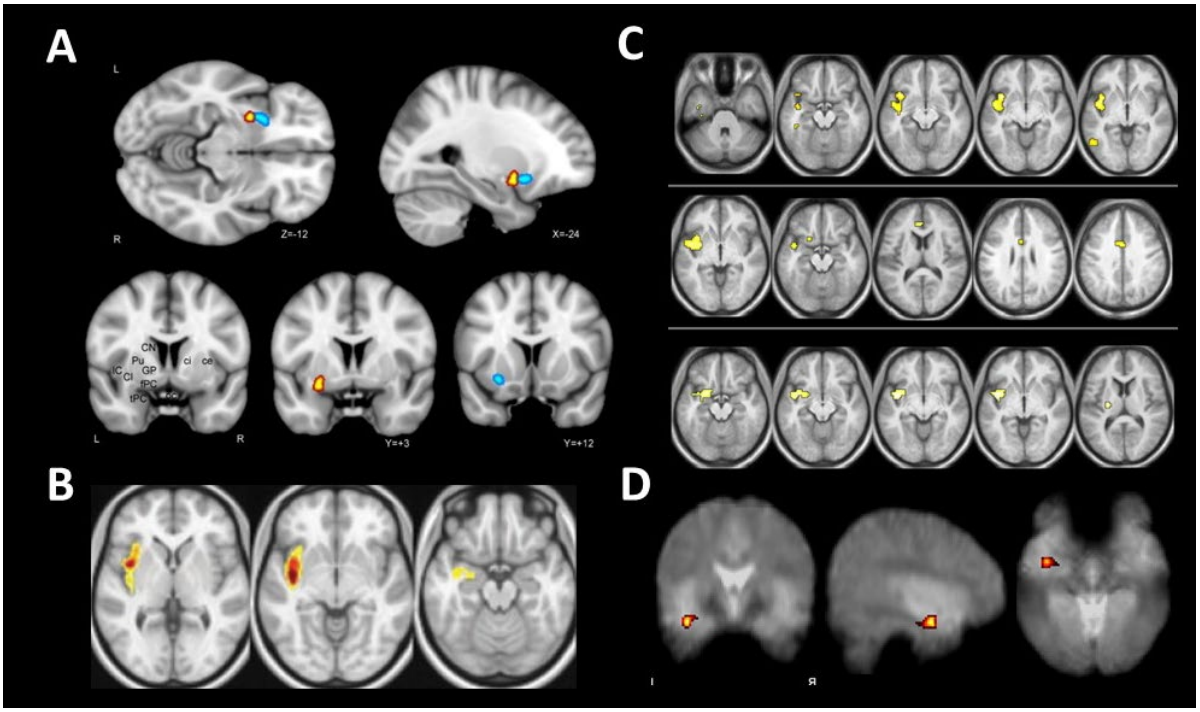
mid-range level of activity (between one and 20 IED per minute) (Salek-Haddadi et al., 2006). As IED occur spontaneously and unpredictably we had to ensure a balanced design by only including patients with a similar number of IED (Friston et al., 2005; Laufs et al., 2007).

To test for any common patterns across the group of patients, we used a random effects model to identify any typical responses consistent across patients (Friston et al., 1999b) and to test the hypothesis of activation in the region of the presumed *area tempestas*. Accordingly, bilateral 0.7 cm x 0.7 cm x 0.7 cm search volumes (2744 mm^3) were each centred between the tip of the temporal pole and the orbitofrontal gyrus based on the aneurysm case report of Mizobuchi et al. (Mizobuchi et al., 1999). FMRI signal changes within in these regions were considered significant at $P<0.05$ corrected for multiple comparisons. In addition, positive responses were explored across the whole brain at a significance threshold of $P<0.001$ (uncorrected at the voxel level).

We found a significant correlation between IED and haemodynamic response ($p<0.05$ corrected for multiple comparisons) common to all 19 patients in an area in the vicinity of the deep anterior piriform cortex, adjacent to putamen and claustrum on the same side as the presumed cortical epileptic focus

Our findings (Laufs et al. , 2011) were replicated in three large studies from Canada (Fahoum et al. 2012), Australia (Flanagan et al. 2014) and Brazil (Coan et al (2014). (figure 1) All these studies found a common brain region on fMRI that was recruited during epileptiform discharges despite having variable seizure foci including both temporal lobe epilepsy and extratemporal focal epilepsy patients. The brain area common to focal epilepsy patients in all of these studies was the ipsilateral piriform cortex.

Patients with extra-temporal focal epilepsy had common areas of abnormal connectivity in an analysis of task-free MRI. (Pedersen et al., 2016). Decreased regional connectivity was observed in the ventromedial prefrontal cortex, as well as lateral temporal cortices of 14 patients with extratemporal focal epilepsy. Despite heterogenous sites of seizure origin, like in the studies cited above, these patients with focal epilepsies had common areas of abnormality (ReHo and DCw measures), including the ipsilateral piriform cortex, temporal neocortex, and ventromedial prefrontal cortex, thalamus and striatum.



Legend Figure 1:

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B

C

D

Proof of concept: post-operative seizure outcome

A case report of a patient with seizure relapse three years following an initially successful right temporal lobectomy for ipsilateral medial temporal sclerosis, was the first to demonstrate a potential role for the piriform cortex/area tempestas for seizure-relapse, if not completely resected. Interictal EEG–fMRI revealed significant BOLD signal changes over the inferior, basal and lateral temporal and temporooccipital cortices posterior to the resection margin, plus a significant BOLD signal change over the ipsilateral basal frontal region, closely corresponding to the piriform cortex. (K. Garganis et al; 2013)

We determined grey matter volume and the size of the piriform cortex on MRI performed before and after standard anterior 2/3 temporal lobe resection in 107 drug-refractory TLE patients with the aim to correlate the extent of surgical resection with postoperative outcome. (Galovic et al., 2019) The current assumption is that successful epilepsy surgery depends on

the complete removal of the tissue that is involved in seizure generation, and thus, inclusion of the piriform cortex within the temporal lobe resection would provide proof-of-concept for the clinical relevance of this area.

Comparing pre- and postoperative MRI, seizure-free patients showed more pronounced grey matter reductions in the ipsilateral piriform cortex than patients who had postoperative seizures. Having determined the area of significant grey matter differences between groups using VBM, we compared the volumes of the piriform cortex and three other mesiotemporal regions involved in TLE (hippocampus, amygdala, entorhinal cortex): a preoperatively reduced volume of the ipsilateral piriform cortex was significantly ($p=0.02$) associated with postoperative seizures, whereas there was no association for the hippocampus, amygdala, or entorhinal cortex.

Comparing the extent of resection between the two outcome groups, a significantly larger proportion of the piriform cortex had been resected in seizure-free patients compared to the surgically refractory group (83% vs. 52%, $p<0.001$). In contrast, the resected proportion of the hippocampus, amygdala, or entorhinal cortex and the overall resection volume did not correlate with postsurgical outcome.

Resected proportion of the piriform cortex was a good predictor of postoperative seizure outcome in individual patients with an area under the receiver operating characteristics curve (AUC) of 0.80 ($p<0.001$, left AUC 0.82, right AUC 0.77). Only 7% (2/30 patients) became seizure free if less than 50% of the piriform cortex were resected, compared to 57% (44/77 patients) if $\geq$ 50% were removed. Resection of at least half of the piriform cortex increased the odds of complete seizure-freedom by a factor of 19 (95% confidence interval [CI] 4-84).

Figure 2 - here

Discussion

The ipsilateral piriform cortex is likely to constitute an important node in focal epilepsy. This area corresponds in location to the physiologically defined "deep piriform cortex" (or *area tempestas*) from which convulsants initiate temporal lobe seizures (Fornai et al., 2005; Maggio et al., 1993), and blockade of glutamate (Fornai et al., 2005; Millan et al., 1986; Piredda and Gale, 1985; Piredda and Gale, 1986) or application of a GABA agonist in this area (Piredda and Gale, 1986) reduce limbic motor seizures.

Averaging imaging data across a group of patients with different sites of seizure onset eliminates signal changes associated with sites of seizure onset (which varied across the patients), and selectively detects signal changes common to all cases. Inherent to the *group* analysis performed in the studies reported here, it appears that although there is likely to be considerable individual variability in potential “epileptogenic networks”, some areas are common to all networks. The piriform cortex was altered in focal epilepsy subjects, whether temporal or extra-temporal lobe epilepsy and regardless of the functional imaging method used, be it EEG-fMRI, task—free MRI with various regional connectivity analysis methods or FMZ-PET, which images the major inhibitory neurotransmitter system. This region close to human frontal olfactory cortex was active in association with interictial EEG spikes, and benzodiazepine-$GABA_A$ receptor complex expression correlated with seizure frequency.

This region closely corresponds to a zone, referred to as *area tempestas*, which is highly epileptogenic in rodents and non-human primates. Taken together, previous functional imaging findings and our most recent post-operative results provide compelling evidence for a critical epileptic area in the human piriform cortex, i.e. the possible location of a human area tempestas.

The piriform cortex is the most susceptible area for epileptogenic stimulation (Gale 1988, Piredda and Gale, 1985; McIntyre DC and Kelly ME, 2000; Roch C et al, 2002; Vaudano et al, 2012) and a node for the spread of epileptic discharges in TLE (Laufs et al, 2011; Vaughan and Jackson, 2014; Flanagan et al, 2014) Hence, we presume that seizures originating from different parts of the temporal lobe can lead to an extension of the epileptic network into the piriform cortex (Roch et al, 2002, 2007; Loescher and Ebert, 1996) Such a spread of the epileptogenic zone, indicated by a presurgical volume-loss in the ipsilateral piriform cortex, was prognostic of poor postsurgical outcome in this study.

Our findings demonstrate that if the epileptic network in the piriform cortex is not sufficiently disrupted by removing at least half of this area, a patient is almost 20-times more likely to suffer seizures postoperatively. Resection of at least 50% of the piriform cortex is a

prerequisite to achieve postoperative seizure-freedom in most TLE patients. The extent of piriform cortex resection was prognostic of postsurgical seizure-outcome and it explained one third of outcome variability. In line with previous research[20], other mesiotemporal regions were not prognostic. Removal of the piriform cortex was safe regarding neuropsychological and psychiatric postsurgical outcome. Our findings suggest that this area may contribute to seizure modulation and that it may be an attractive target for neurosurgical or pharmacological epilepsy therapy.

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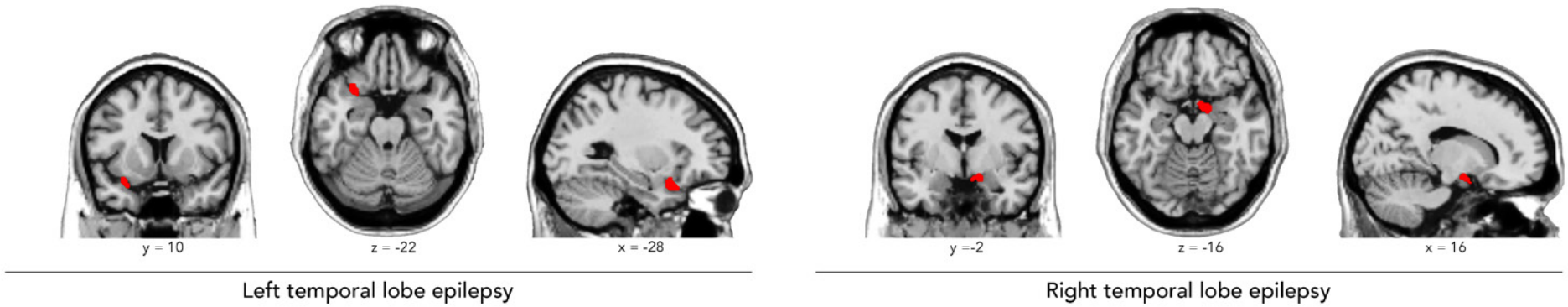
A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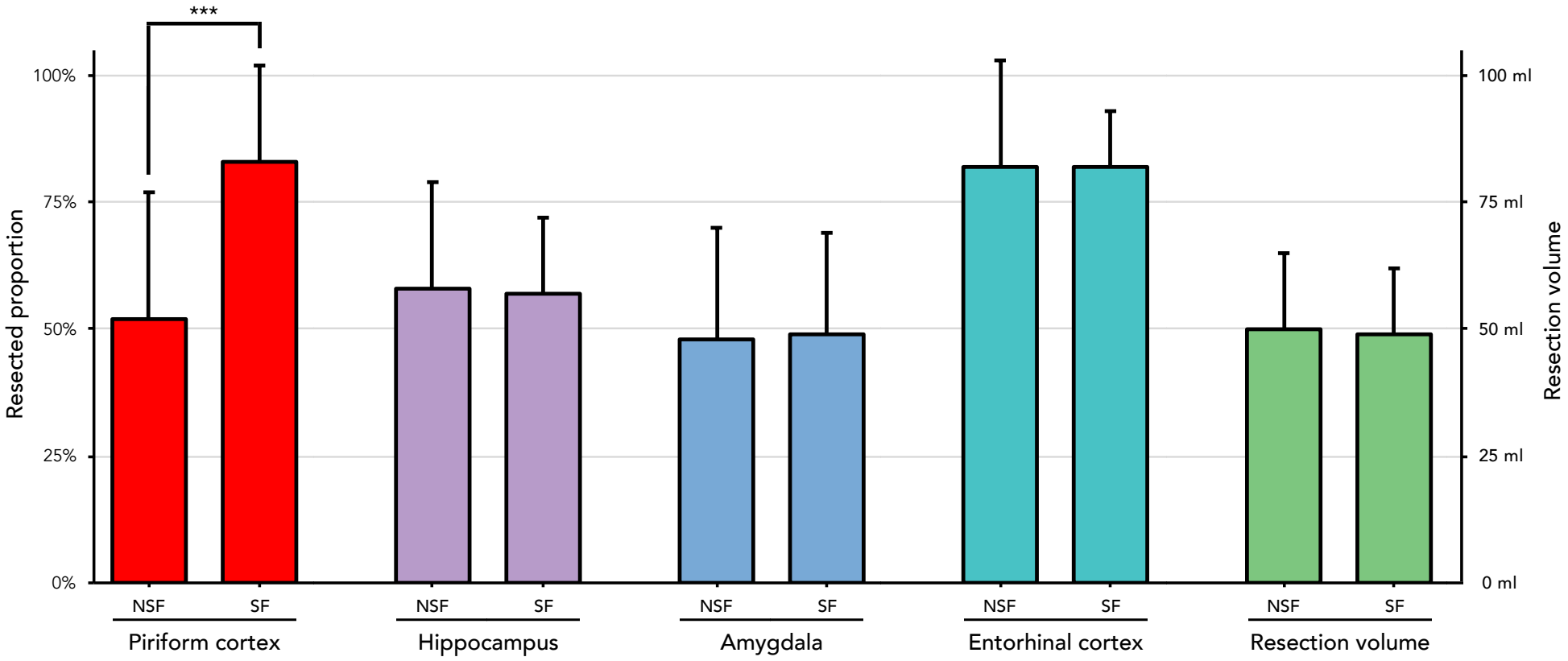
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Entorhinal cortex
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